

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
28 July 2005 (28.07.2005)

PCT

(10) International Publication Number
WO 2005/067977 A1

(51) International Patent Classification⁷: **A61K 47/36**

(21) International Application Number:
PCT/SK2005/000001

(22) International Filing Date: 4 January 2005 (04.01.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PP 34-2004 14 January 2004 (14.01.2004) SK

(71) Applicant (for all designated States except US): **PLEURAN, S.R.O.** [SK/SK]; Platenicka 24, 821 09 Bratislava (SK).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **GABRIZOVA, Leona** [SK/SK]; Nové Zahrady VI/10, 821 05 Bratislava (SK).

(74) Agent: **HOLOUBKOVA, Maria**; Holoubkova Patent and Trademark Agency, Dostojevského rad 1, 811 09 Bratislava (SK).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD OF PREPARATION OF A FUNGAL GLUCANE HYDROGEL HAVING ANTIBACTERIAL AND IMMUNOSTIMULANT ACTIVITY, AND USE THEREOF

(57) Abstract: A method of preparation of a fungal glucane hydrogel having antibacterial and immunostimulant activity by alkaline deproteination and subsequent elimination of water-soluble components consists in that the obtained insoluble glucan is subsequently hydrated by wet grinding at a rotational speed of 3000 to 9000 rpm for 10 to 20 min. to a swelling volume in water of 50 to 500 ml/g. Then it is adjusted by heat sterilization at a temperature of 90 to 110 °C for 20 to 30 min., what results in a gel which is formed by fungal polysaccharide with the β -(1,3)-D-bond in the principal chain, with a concentration of 0.5 to 3 % by weight. The fungal glucane hydrogel may be utilized for preparation of cosmetical, pharmaceutical and foodstuff products.



WO 2005/067977 A1

Method of preparation of a fungal glucane hydrogel having antibacterial and immunostimulant activity, and use thereof.

Technical Field

Present invention relates to a method of preparation of a fungal glucane hydrogel having antibacterial and immunostimulant activity, and to its use.

Background Art

Some of the natural polysaccharides are characterized by immunostimulant and other pharmacological properties. The main carrier of the immunostimulant activity in natural polysaccharides are those polysaccharides, which have the β -(1,3)-D-glycosidic bond in the principal polysaccharide chain. It has been proven that immunoglucanes enhance immunity against various bacterial and virus diseases, they exhibit anticancer activity, potentiate the effect in radiotherapy and chemotherapy of of oncological patients.

Immunostimulant polysaccharides exist in cell walls of bacteria, yeasts and several fungi, especially of the Basidiomycetes genus. Immunopharmacologically active substances, to which the β -(1,3)-D-glycanes belong, are able to nonspecifically modify an extensive set of bacterial, fungal, parasitical and virus diseases. The mechanism of glucan action differs considerably from that of chemotherapeutics and antibiotics.

An immunostimulant glucan is according to the SK patent No. 282870 isolated from fruiting bodies of oyster mushroom, preferably from its stems by defibering, subsequent bleaching with hydrogen peroxide at a temperature of 15 to 25 °C for 15 to 24 hours in a medium of sodium hydroxide solution. The defibering is performed within 26 hours after picking the oyster mushrooms, which are stored at a temperature of 4 to 8 °C, in a medium of an at least double amount of aqueous sodium or potassium carbonate solution with a concentration of 0.05 to 0.15 % by weight, pH of the solution being 8 to 9, for 1 to 8 minutes. This results in a reaction suspension with the enzymatic activity of the present β -(1,3)-D-glucanase eliminated. From this suspension glucan is obtained by filtration and thorough washing. The glucan is then squeezed, dehydrated with ethanol and dried.

The aim of the present invention is recovery of glucan in the form of hydrogel, which is more active than the so far prepared glucan in the form of a powder.

Disclosure of Invention

The subject-matter of preparation of fungal glucan hydrogel having antibacterial and immunostimulant activity by alkaline deproteination and subsequent elimination of water-soluble components consists in that the so obtained insoluble glucan is subsequently hydrated by wet grinding at a rotational speed of 3000 to 9000 rpm for 10 to 20 min. to a swelling volume of 50 to 500 ml/g. The mixture prepared in this way is adjusted in the form of gel by heat sterilization at a temperature of 90 to 110 °C for 20 to 30 min., what results in a fungal polysaccharide with the β -(1,3)-D-bond in the principal chain, with a concentration of 0.5 to 3 % by weight. If the so prepared hydrogel is used immediately after preparation, it needs not to be sterilized. However, in the case of longer storage it is chemically sterilized by addition of 0.02 % of benzoic acid.

An aqueous solution of fungal glucan hydrogel is tasteless and odourless, it contains in ash at most 1.0 % of nitrogen, at most 1.7 % of sulfur, and according to the Foodstuff Code it is microbiologically harmless.

The fungal glucan hydrogel may be used for preparation of cosmetic formulations, where auxiliary substances forming hydrophilic ointment base are added to the fungal glucan hydrogel. Among the cosmetic formulations there are preparations for acne treating, for regeneration of skin, which is ageing, damaged by solar radiation and extremely stressed.

For pharmaceutical purposes it is used at various, including more serious skin injuries, like crus ulcers, eczema, inflammations, decubiti treating, and for accelerating of wounds and scars healing after surgical intervention, because the fungal glucan hydrogel is, besides having immunostimulant properties, also very well applicable to the skin.

The fungal glucan hydrogel is also suitable for preparation of food products, like yoghurts, curdy spreads, nutritional supplements for children, as it enhances immunity of the human organism.

Examples of Embodiments

Example 1

100 g of glucane, prepared from fruiting bodies of oyster mushroom (*Pleurotus ostreatus*) is hydrated in a high-speed mixer in 5 l of water for 20 minutes at the rotational speed of 6000 rpm. Under these conditions, glucan is hydrated to the swelling volume in water of 250 ml/g, and the resulting viscous gel is subsequently heat sterilized at a temperature of 110 °C for 20 min.

Example 2

5 kg of fungal glucan hydrogel, prepared according to Example 1, is homogenized in a homogenizer with 10 kg of ointment base AMBIDERMANN, with addition of chemosterilant, N-cetyl-N,N,N-trimethylammonium bromide, in an amount of 0.1 %, referred to the weight of the resulting cream, suitable for dermal applications at various diagnoses of skin diseases or to surgical wounds and skin defects.

Example 3

1 kg of fungal glucan is hydrated in 50 l of water in a high-speed mixer for 30 minutes at the rotational speed of 6000 rpm. Then 200 g of citric acid, 40 g of benzoic acid and 40 kg of fructose are added and it is sterilized at a temperature of 110 °C for 20 min. After sterilization 50 l of sterile water are added, in which 1 kg of Ca-ascorbate has been dissolved. After homogenization of both components microbially stabilized syrup is obtained and filled in 100 ml glass vials.

The resulting syrup contains 10 mg of glucan hydrogel in 1 ml of syrup. The product is suitable especially for children in prophylactic and therapeutic treatment against various children's diseases.

Example 4

The glucan hydrogel in an amount of 100 mg/l is fed in a 1000 l tank with milk before milk sterilization. After sterilization and cooling the glucan hydrogel is inoculated with yoghurt culture in a regular technological process. The yoghurt

produced contains 10 mg of immunoglucan in 100 ml of yoghurt. It is suitable especially for infant food and gerontologic population.

Industrial Applicability

The fungal glucan hydrogel is suitable for preparation of products having antibacterial and immunostimulant properties in foodstuff and pharmaceutical industry. It is also suitable for preparation of creams for various purposes in cosmetic industry, as it exhibits good application properties.

CLAIMS

1. Method of preparation of a fungal glucane hydrogel having antibacterial and immunostimulant activity by alkaline deproteination and subsequent elimination of water-soluble components, characterized in that the obtained insoluble glucan is subsequently hydrated by wet grinding at a rotational speed of 3000 to 9000 rpm for 10 to 20 min. to a swelling volume in water of 50 to 500 ml/g, and finally it is adjusted by heat sterilization at a temperature of 90 to 110 °C for 20 to 30 min., what results in a gel which is formed by fungal polysaccharide with the β -(1,3)-D-bond in the principal chain, with a concentration of 0.5 to 3 % by weight.
2. Method according to claim 1, characterized in that the insoluble glucan is prepared from fruiting bodies of oyster mushroom (*Pleurotus ostreatus*).
3. Method according to claims 1 and 2, characterized in that the resulting gel is formed by fungal polysaccharide with the β -(1,3)-D-bond branched at every fourth anhydroglucose unit.
4. Method according to claims 1 to 3, characterized in that the resulting fungal glucane hydrogel is chemically sterilized by addition of 0.02 % of benzoic acid.
5. Use of fungal glucane hydrogel according to preceding claims for preparation of cosmetical, pharmaceutical and foodstuff products.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SK2005/000001

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| X | US 4 774 093 A (PROVONCHEE ET AL) 27 September 1988 (1988-09-27) column 1, line 15 - line 35 column 5 - column 6; example 1 ----- | 5 |
| X | EP 0 875 244 A (CIBA SPECIALTY CHEMICALS HOLDING INC) 4 November 1998 (1998-11-04) page 4, line 11 - line 42 page 5; example 3 ----- | 5 |
| X | US 5 158 772 A (DAVIS ET AL) 27 October 1992 (1992-10-27) columns 10-12; examples 1,2 claims 1-6 ----- | 5 |
| | -/-- | |

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

27 June 2005

Date of mailing of the international search report

04/07/2005

Name and mailing address of the ISA

European Patent Office, P.B 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Muller, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/SK2005/000001

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X | WO 94/03500 A (CONSIGLIO NAZIONALE DELLE RICERCHE; ISTITUTO SUPERIORE DI SANITA; CONS) 17 February 1994 (1994-02-17) abstract page 16, line 12 - line 14 claims 1-8 | 5 |
| A | ----- WO 02/085950 A (GABRIZOVA, LEONA) 31 October 2002 (2002-10-31) the whole document ----- | 1-5 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/SK2005/000001

| Patent document cited in search report | | Publication date | Patent family member(s) | Publication date |
|-------------------------------------------|---|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| US 4774093 | A | 27-09-1988 | DE 3621303 A1 GB 2176795 A ,B JP 62030102 A SE 8602805 A | 08-01-1987 07-01-1987 09-02-1987 26-12-1986 |
| EP 0875244 | A | 04-11-1998 | AU 737154 B2 AU 6376298 A BR 9801517 A CN 1198323 A CZ 9801329 A3 EP 0875244 A2 ID 19802 A JP 11000190 A US 6162447 A ZA 9803660 A | 09-08-2001 05-11-1998 11-01-2000 11-11-1998 11-11-1998 04-11-1998 06-08-1998 06-01-1999 19-12-2000 02-11-1998 |
| US 5158772 | A | 27-10-1992 | NONE | |
| WO 9403500 | A | 17-02-1994 | IT 1256035 B AU 4706393 A CN 1082056 A WO 9403500 A1 EP 0654047 A1 MX 9304793 A1 ZA 9305730 A | 21-11-1995 03-03-1994 16-02-1994 17-02-1994 24-05-1995 31-05-1994 03-03-1994 |
| WO 02085950 | A | 31-10-2002 | SK 5482001 A3 CZ 20031404 A3 HU 0303916 A2 WO 02085950 A1 | 06-11-2002 13-08-2003 29-03-2004 31-10-2002 |